# PERFORMANCE OF EUROPEAN ASPEN (*Populus tremula* L.) UNDER THE COMBINED EFFECT OF ELEVATED TEMPERATURE AND UV RADIATION

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## ABSTRACT

Elevated temperature stimulates the physiology and growth of trees in the boreal region in general whereas the effects of elevated UVB radiation can be positive, negative or neutral. This field experiment was conducted to investigate the effects of elevated temperature and UV radiation, separately and interactively on the chlorophyll content, gas exchange and growth performance of twelve European aspen (*Populus tremula*) genotypes including six females and six males. A modulated system of 2˚C elevated temperature and 32% enhanced UV radiation above the ambient levels were applied to the plants. In 2013, exposure period for the two-year-old plants was between the early June and mid-September. Chlorophyll content and gas exchange parameters were measured twice in early July and mid-August, and growth parameters were measured six times at about three weeks interval. Elevated temperature increased chlorophyll content, photosynthesis, stomatal conductance and transpiration while substomatal $CO_2$ was decreased. Elevated temperature also increased height, basal diameter and shoot biomass. On the other hand, the main effects of elevated UV radiation were not significant on all the studied parameters except the marginally significant positive effect on shoot biomass. The combined effects were not significant on any of the measured parameters, but elevated UVB radiation mitigated to some extent the stimulation effects of elevated temperature on almost every parameter studied. Chlorophyll content, gas exchange and growth parameters were varied according to genotypes except the transpiration. This genotype-depending variation was further influenced by elevated temperature and UV radiation in height and basal diameter growth. The main effects of gender were only significant for chlorophyll content and height growth. Female genotypes had higher chlorophyll content whereas male genotypes had higher height growth. Genotypes from Southern Finland showed higher growth performance over the genotypes of Eastern Finland. In future, experiments with more European aspen genotypes may ensure the more accurate results of genotypic variation.

## ACKNOWLEDGEMENTS

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# 1 INTRODUCTION

Many studies have confirmed global climate change over the last century (e.g. IPCC 2007, 2013). Elevated temperature is among the major consequences of global climate change. Global mean surface temperature has been enhanced by 0.85˚C from 1880 to 2012 and is predicted to the increase of 0.3-4.8˚C by the end of current century (IPCC 2013). In northern Europe, temperature is expected to increase more than the global mean and in Finland, it is projected that the annual mean temperature will rise by 2-7 ˚C by the year 2080 (Jylhä et al. 2004). Numerous studies have suggested that elevated temperature increases the rate of photosynthesis in broad leaf tree species at high latitude (Mäenpää et al. 2011, Hartikainen et al. 2012) while some studies revealed that the excessive heat stress decreases the photosynthesis (Georgieva et al. 2000, Velitchkova et al. 2013).

Moreover, elevated temperature affects stomatal conductance, transpiration rate, substomatal $CO_2$ and chlorophyll content of leaves (Ormrod et al. 1999, Zhao and Liu 2009, Yan et al. 2013). Stomatal conductance in turn controls transpiration, regulates substomatal $CO_{2,}$ and thereby photosynthetic carbon gain (Jones 1998, Lombardozzi et al. 2012). Though chlorophyll content does not always correspond to photosynthetic rate (Granti et al. 1998), some studies found the positive correlation between them (Naidu & Swamy 1995, Proietti 1998). Therefore, the effect of elevated temperature on photosynthetic performance can be further manipulated by other physiological factors and foliar pigments. In addition to the aforementioned consequences, elevated temperature increases the height, diameter and biomass of broad leaf tree species in the boreal regions (Veteli et al. 2002, Nybakken et al. 2012, Way et al. 2013) until the optimum temperature is reached. Actually, increasing the photosynthetic rate results in faster growth and higher biomass production since photosynthetic carbon gain accounts for 90-100% of plant biomass accumulation (Poorter et al. 1990, Abdul-hamid & Mencuccini 2008).

On the other hand, although the emissions of ozone destroying substances is strictly controlled at high latitudes in northern countries (IPCC 2007), the recovery of the stratospheric ozone layer will take several decades and depends on other factors including aerosols, clouds, etc (Randriamanana et al. 2014a). The thinned stratospheric ozone layer permits the higher amount of solar ultraviolet-B radiation (UV between 280 nm and 315 nm) reaching the earth surface. UVB radiation is also an important ecophysiological factor influencing plant growth and photosynthesis (Caldwell et al. 2007, Ballare et al. 2011). Experimental studies, however, found that plants respond differently to UVB radiation. Some

of the studies reported positive effects of UVB radiation on chlorophyll content (Niemi et al. 2002) while others found negative effects (Robinson et al. 2005, He et al. 2006), and no effects (Lud et al. 2001). Similarly, some experiments found increasing (Li et al. 2010), decreasing (Wand et al. 1996, Li et al. 2010), and neutral (Antonelli et al. 1997, Lud et al. 2001, Niemi et al. 2002, Robinson et al. 2005) effects of elevated UVB on gas exchange parameters. There has been variation in plant growth and biomass also as a consequence of UVB effects, with increasing in some studies (Lavola et al. 2000, Kyparissis et al. 2001), decreasing (Antonelli et al. 1997, Day et al. 2001, Xiong & Day 2001) and no effects at all in others (Wand et al. 1996). Methodologies used for UVB radiation in different studies may be one of the reasons for the variation in results (Newsham & Robinson 2009). However, UVB radiation effects can be species-specific also (Björn et al. 1997, Gwynn-Jones et al. 1997).

Apart from the environmental factors, genotypic characters also regulate the physiology and growth of trees (Lamhamedi et al. 2000, Aspinwall et al. 2010). Therefore, some of the genotypes of a species have higher growth potential compared to others as result of genetic variation. In a field experiment, 5 *Populus* genotypes of 3 different parentages showed significant variation in volume, woody biomass, net photosynthesis and total leaf area (Barigah et al. 1994). In a closed-top chamber study with elevated $CO_2$, temperature and UVB radiation revealed the significant variation in height, diameter and biomass among the dark-leaved willow (*Salix myrsinifolia*) genotypes. This genotype-dependent variation in growth parameters was further influenced by climatic factors (Paajanen et al. 2011).

It seems from the above predictions (Jylhä et al. 2004, IPCC 2013, Randriamanana et al. 2014a) that future climatic conditions will differ from the present conditions and boreal forest trees have to adapt to the new climatic scenario. European aspen (*Populus tremula*) is a dioecious tree species and belongs to the *Salicaceae* family. In northern Europe, it is the only native *Populus* species. In fact, it is a keystone species in boreal forest biodiversity. In Finland, more than 200 other species depend on it as a source of food or as a habitat (Siitonen 1999, Kouki et al. 2004). However, the interaction studies of European aspen to the divergent climatic conditions are really scarce. Besides, majority of the climate change studies have been conducted in greenhouses or chambers (e.g. Koti et al. 2007, Lavola et al. 2013). There are some shortcomings in the experiments carried out in these kinds of conditions. In the growth chambers and greenhouse experiments, often supplied unrealistically high ratio of UVB to photosynthetically active radiation (PAR; 400-700 nm) exaggerates the effect of UVB as a result of insufficient PAR (Kostina et al. 2001). In addition, plants do not experience unpredictable variations in light, temperature, humidity, soil moisture and wind in

the controlled environment (Jänkänpää et al. 2012). These kinds of problems can be avoided in a field condition which may produce more realistic conclusions.

It is predicted and clear that different climatic factors would increase simultaneously. Therefore, elevated temperature might compensate the damaging effects of elevated UVB radiation. However, Day et al. (1999) did not find any interaction effects on the growth, reproduction, chlorophyll content and UV-absorbing pigments of two vascular plants. Nybakken et al. (2012) also reported no combined effects on the height, basal diameter and shoot biomass apart from the significant interactive effects on leaf area and some phenolic compounds of *S. myrsinifolia.* In the present study, twelve genotypes of European aspen were exposed to modulated and elevated temperature and UV radiation in an open field to obtain a better understanding of the impact of climatic change on this species, and especially on its genotypes.

## 2 AIMS AND HYPOTHESES

The main aim of this study was to assess the combined effects of elevated temperature and UV radiation on the gas exchange parameters and growth performance of two-year-old European aspen in a field condition. Moreover, I aimed to examine the response of different genotypes of European aspen to the increased temperature and UV radiation. The measurements were taken about chlorophyll content index, gas exchange parameters (photosynthetic rate, stomatal conductance, transpiration rate and substomatal $CO_2$), height, basal diameter and shoot biomass. For the completion of this work, the following hypotheses were evaluated:

**1.** Elevated temperature would increase the chlorophyll content, gas exchange and growth of European aspen.
**2.** Elevated UV radiation would reduce the chlorophyll content, gas exchange and growth of European aspen.
**3.** In a combined treatment, elevated UVB radiation would counteract the stimulation effects of elevated temperature.
**4.** Moreover, there would be genotype-specific differences in all of these responses, and these genotypic variations would be further influenced by the elevated temperature and elevated UV radiation.

# 3 MATERIALS AND METHODS

## 3.1 Location of the study area and experimental design

The study area (Fig. 1) is located in a field inside the botanical garden of Joensuu, eastern Finland (62˚35′N, 29˚46′E). Experimental plots were prepared in the beginning of growing season 2012 by adding a 10 cm layer (336 L in each plot) of Biolan Black Mold (mineral soil) on the top of the original field soil. Biolan Black Mold contained three different nutrients with a concentration of 120 mg nitrogen (N), 90 mg phosphorus (P) and 600 mg Potassium (K) per liter. The experimental design was according to Nybakken et al. (2012). Six different combinations of treatments were applied in this experiment. They were control (C), elevated UVA (UVA), elevated UVB (UVB), elevated temperature (T), UVA+T and UVB+T. Six replicates of each treatment (total 36 plots) placed randomly in the field. Each plot size was 1.2 m x 2.8 m. Effective area for growing plants was about 0.80 m x 2.40 m within each plot. The experimental area was surrounded by a 1.5 m high fence for the security reasons and to protect plants from large herbivores. Moreover, a metal shelter of 2 mm thickness was set underneath the fence to prevent vole intrusion.


**Fig 1.** Picture of the experimental field in summer 2013 (photo Riitta Julkunen-Tiitto)

## 3.2 Temperature and UV radiation exposure to the plants

A modulated system of elevated temperature and elevated UV radiation were applied in the field. Temperature enhancement was set to 2˚C above the ambient level in the study area. On the other hand, UVB radiation was aimed to be increased by 32% above the ambient level which corresponds to about 20% reduction in ozone layer above central Finland (Björn 1990). In each plot, one aluminum frame (1.5 m wide and 3.0 m long, effective area was 0.80 m × 2.40 m) was mounted above the canopy. The frames were 3 m apart from each other in all directions. Six 40 W fluorescent UV-lamps (1.2 m long, UVB-313, Q-panel Co, Cleveland, OH) were installed on each frame following a 'cosine' distribution (Björn 1990) to confirm uniform radiation dose inside the plot. The lamps were kept 60 cm above the top canopy of the plants. Cellulose diacetate filters (0.115 mm, Kotelorauma, Finland) were wrapped around the UVB lamps to attenuate any radiation below 290 nm. In case of UVA lamps, polyester films (0.175 mm, Kariplast, Finland) were wrapped to absorb the radiation of below 315 nm. In the control plots, un-energized lamps (sunlight controls) were installed in the frames to equalize the shading as in treatment plots. Every third week the filters were replaced by the new ones. On the other hand, temperature treatment was realized using infrared (IR) heaters (CIR 105, FRICO, Partille, Sweden). Two IR-heaters were installed in the middle of the temperature treatment frames, one after another. In the temperature control plots, a wooden piece of same size as IR-heaters was installed in the frames in the same way to equalize the shading as in the treatment plots. A metal post was used in every plot to fix the aluminum frames. The frames were lifted upwards in every third week to maintain the distance of about 60 cm between the IR radiators and the highest shoot.

Self-made linear temperature sensors with four PT1000 probe elements each having a connection cable were used to record the temperature. Two probe elements were fixed above the control frames for ambient values whereas two others were installed under the temperature frames for set-point elevated values. Thies Clima (Göttingen, Germany) sensors were used to measure UVB radiation in the field. Two sensors were used to measure the ambient values above the control frames while two were used for the set-point elevated UVB values below the UV tube frames. The sensors measure radiation between 250 and 325 nm with a peak at 300 nm. The maximum signal of the sensors is 20 mA which is equivalent to a UVB radiation intensity of 500 mW. Modulator software (IPC100 configuration program, and e-console measuring and data saving program) was used to calculate the set-point values. This software also controlled the intensity of the UV lamps and IR radiators. The soil

temperature was recorded under the same frames where the control and the elevated temperature were logged. In 2013, treatments were started in 5th June and ended in 13th September.

## 3.3 Plant material

For this experiment, twelve genotypes including six females (gt1-6) and six males (gt7-12) of European aspen were used. Plant materials were collected from about 30-40 years old trees located in different places in Finland (see the following Table 1). Five individual plants of each genotype, replicated by micropropagation were randomly planted in each plot (60 plants/plot in total) in five rows in June, 2012. The distance between two consecutive plants in a plot was 25 cm to all directions.

**Table 1.** Site for parent tree

| Genotype | Gender | Location of parent tree | Province | Latitude (N)/longitude (E) |
|---|---|---|---|---|
| gt1 | Female | Pieksämäki | Eastern Finland | 62º18´/27º07´ |
| gt2 | Female | Loppi | Southern Finland | 60º43´/24º27´ |
| gt3 | Female | Loppi | Southern Finland | 60º43´/24º27´ |
| gt4 | Female | Loppi | Southern Finland | 60º43´/24º27´ |
| gt5 | Female | Pieksämäki | Eastern Finland | 62º18´/27º07´ |
| gt6 | Female | Polvijärvi | Eastern Finland | 62º52´/29º19´ |
| gt7 | Male | Kaavi | Eastern Finland | 62º54´/28º42´ |
| gt8 | Male | Loppi | Southern Finland | 60º43´/24º27´ |
| gt9 | Male | Loppi | Southern Finland | 60º43´/24º27´ |
| gt10 | Male | Liperi | Eastern Finland | 62º41´/29º33´ |
| gt11 | Male | Kontiolahti | Eastern Finland | 62º38´/29º41´ |
| gt12 | Male | Polvijärvi | Eastern Finland | 62º52´/29º19´ |

## 3.4 Chlorophyll content, gas exchange & growth measurements

Chlorophyll content, gas exchange and growth measurements were taken from the two-year-old European aspen plants. During the growing season of 2013, four plots under each treatment of C, T, UVB, and UVB+T were selected randomly for chlorophyll content and gas exchange measurements. No plots under UVA and UVA+T treatments were considered for the measurements of chlorophyll content and gas exchange parameters. This is because of previous studies found no significant differences in chlorophyll content and gas exchange parameters between the plants under UVA and control, and between the plants under T and UVA+T (Randriamanana et al. unpublished data). In each selected plot, one individual from

each genotype was selected randomly for the measurement of chlorophyll content and gas exchange parameters. Measurements were taken between 9:00 o'clock and 15:00 o'clock. Chlorophyll content index (CCI) and gas exchange parameters including photosynthetic rate (A), substomatal $CO_2$ (Ci), stomatal conductance of $CO_2$ ($g_s$) and transpiration rate (E) were measured twice (09-16 July and 20-27 August) during the growing season. During the second measurement time, gt6 was not considered for the measurement because of no availability of healthy leaves in it. CCI was measured with a CCM-200 chlorophyll meter (Opti-Sciences, Tyngsboro, MA, USA). The instrument uses calibrated light emitting diodes (LEDs) and receptors to calculate CCI. The LEDs emit specific wavelengths in the red (653 nm) and infrared (931 nm) ranges and the receptors calculate the ratio of percent transmission of the two wavelengths through a leaf sample which gives CCI, a relative value of the chlorophyll content of leaves and proportionate to the amount of chlorophyll to the sample (Apogee 2014). In each randomly selected individual, one youngest mature leaf was measured, not affected by any disease and by avoiding major vein. In each leaf, two measurements including one from the left side and another one from the right side of the central vein were taken. For analysis, average of the two values was used.

Leaf gas exchange parameters (A, Ci, gs and E) were also measured from the same leaves as used for CCI measurement. These parameters were determined with a portable photosynthesis system LCpro+ (ADC BioScientific Ltd., Hertfordshire, UK). The LCpro+ is an open-system Infra Red Gas Analyzer (IRGA) which facilitates ambient fresh air to pass through the plant leaf chamber and measures gas exchange of leaves. Before the measurements, calibrations for flow meter was made and $CO_2$ reference (Cref) and $CO_2$ analysis (C'an) were stabilized to obtain similar $CO_2$ levels (^C = 0). Moreover, chamber temperature and Photosynthetic Active Radiation (PAR) were set at 25 ˚C and 1200 µmol $m^{-2}$ $s^{-1}$, respectively. The saturating level of PAR was fixed based on light curve measurements where photosynthetic rates were checked at different levels of irradiance: 300, 600, 900, 1200, 1500, 0 and 100 µmol $m^{-2}$ $s^{-1}$. The photosynthetic rate plateaued at 1200 µmol $m^{-2}$ $s^{-1}$. In each measurement, parameter values were recorded after at least two minutes of leaf acclimation within the measurement chamber.

Height of the longest shoot and basal diameter of all experimental plants were measured six times at approximately three weeks interval from late May to early September 2013. The plants were marked 1 cm above the root collar with a marker pen at the beginning of growing season. At every time, diameter was measured at the marked point by using a vernier caliper. The height was measured with a measuring stick from the root collar to the

tip of the longest shoot. At the end of the growing season, one individual of each genotype from each plot was harvested for biomass measurement. After that they were dried at the room temperature in paper bags and weighed.

## 3.5 Statistical analyses

The effects of temperature, UV and genotype and their interactions on the photosynthetic parameters and growth were examined by linear mixed effects model using IBM SPSS Statistics for Windows (Version 19.0. Armonk, NY: IBM Corp). Temperature, UV, genotype and measurement date were used as fixed factors and plot as a random factor. For the chlorophyll content, gas exchange parameters, height and diameter growth, measurement date was set as a repeated variable since they were measured more than once and the first measurement values of these parameters were set as covariates in linear mixed effects model with repeated measures. Moreover, when more than one individual from each genotype from one frame was measured for photosynthetic parameters, the mean value for these individuals was used in the statistical analysis. Normality of all the variables was checked. CCI was square root-transformed and shoot biomass was log-transformed to ensure the normal distribution of data. The residuals were also checked for the normality.

# 4 RESULTS

## 4.1 Chlorophyll content index (CCI)

The effect of temperature on the CCI of two-year-old European aspen was statistically significant (Table 2). CCI was increased by 28% under elevated temperature (Fig. 2B) and 22% under UVB + T (Fig. 2D) as compared to the control treatment (Fig. 2A). Though CCI was higher under UVB + T in comparison with the control plants, elevated UVB actually decreased CCI which was demonstrated by 7% reduction under UVB treatment (Fig. 2C). However, the decreasing effect of elevated UVB on CCI was not statistically significant (Table 2). Genotypes differed significantly in their CCI (Table 2) since CCI was comparatively higher in gt3, gt5, gt6 and gt1 than the others when averaged across the treatments (Fig. 2). This genotype-dependent variation in CCI became even higher through time and under UVB+T, which was indicated by the statistically significant interaction of UV x Time x Genotype and statistically marginally significant interaction of T x UV x Time x Genotype (Table 2). Female genotypes had 9% higher CCI than their male counterparts. Therefore, the effect of gender on CCI was statistically significant (Table 2).

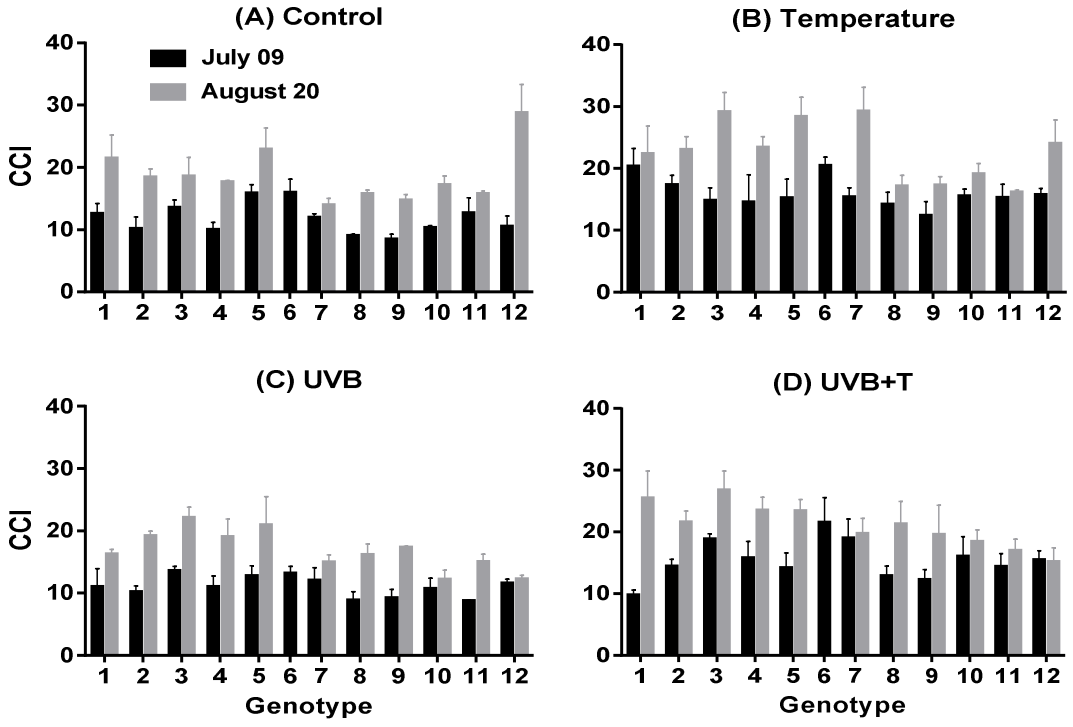


**Fig 2.** Chlorophyll content index (CCI) (mean± SE) of European aspen genotypes grown under (A) control, (B) temperature, (C) UVB and (D) UVB + T.

**Table 2.** F-values obtained from the linear mixed model analysis of the effects of enhanced temperature and UV on CCI, A, Ci, $g_s$ and E in European aspen (***, *P < 0.001;* **, *P < 0.01;* *, *P < 0.05;* MS (marginally significant), *P < 0.1*). CCI was square root-transformed to meet normality assumptions.

| Effect | CCI | A | Ci | $g_s$ | E |
|---|---|---|---|---|---|
| T | 17.202** | 5.005* | 5.509* | 0.010 | 0.425 |
| UV | 0.903 | 0.553 | 1.341 | 0.018 | 0.126 |
| Time | 227.967*** | 0.466 | 2.014 | 24.084*** | 86.746*** |
| T x UV | 0.017 | 0.010 | 2.019 | 2.391 | 0.011 |
| T x Time | 0.681 | 4.063* | 1.711 | 37.306*** | 29.078*** |
| UV x Time | 0.866 | 8.382** | 5.441* | 2.379 | 3.023$^{MS}$ |
| T x UV x Time | 0.181 | 5.499* | 0.011 | 9.889** | 69.726*** |
| Sex | 34.974*** | 0.122 | 1.507 | 1.734 | 0.709 |
| Genotype | 7.441*** | 2.570** | 2.410** | 1.922* | 0.229 |
| T x Genotype | 0.888 | 0.937 | 0.680 | 0.926 | 1.385 |
| UV x Genotype | 1.968* | 1.816$^{MS}$ | 1.530 | 1.254 | 0.898 |
| Time x Genotype | 1.518 | 1.644$^{MS}$ | 1.246 | 1.701$^{MS}$ | 1.576 |
| T x UV x Genotype | 0.461 | 1.336 | 1.030 | 0.763 | 1.067 |
| T x Time x Genotype | 1.156 | 0.538 | 0.424 | 0.971 | 0.748 |
| UV x Time x Genotype | 2.656** | 0.499 | 0.793 | 0.680 | 0.899 |
| T x UV x Time x Genotype | 1.871$^{MS}$ | 0.803 | 0.893 | 0.479 | 0.277 |

## 4.2 Photosynthetic rate (A)

There was a statistically significant effect of temperature on A (Table 2). A was 6% higher under enhanced temperature (Fig. 3B) but 4% higher under enhanced UVB + T (Fig. 3D) when compared to control plants (Fig. 3A). The main effect of UV on A was not statistically significant (Table 2). However, the interaction between temperature, UV and time was statistically significant (Table 2). Hence, in the mid-August as compared to early July, A was increased by 15% under elevated temperature (Fig. 3B) and decreased by 8% under both UVB (Fig. 3C) and UVB + T (Fig. 3D). Moreover, some of the genotypes (gt8, gt1, gt7 and gt11) had comparatively higher A than the others based on the average values over all the treatments (Fig. 3). Therefore, statistically significant difference in A was found across the genotypes (Table 2). The interaction of UV x Genotype was also statistically marginally significant (Table 2) indicating that some of the genotypes were more affected than the others by elevated UVB. In addition, the interaction between genotype and time was statistically marginally significant (Table 2) which is the result of increasing genotype-dependent variation in A in the mid-august in comparison with early July (Fig. 3). There was no statistically significant gender differences in A (Table 2).

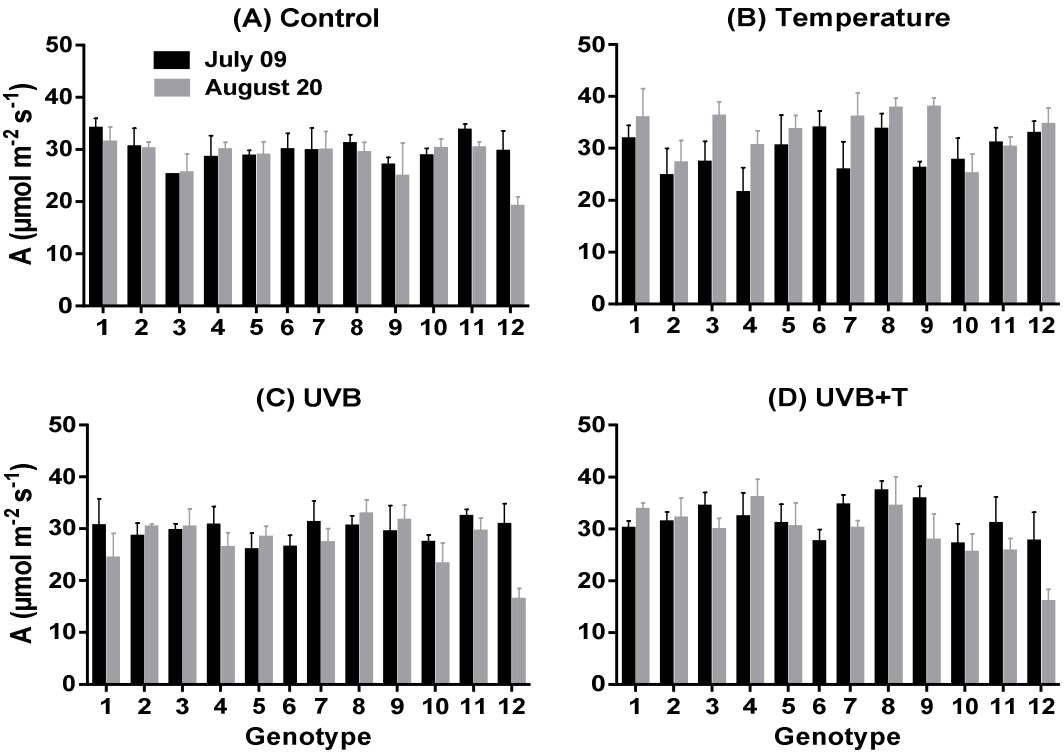


**Fig 3.** Photosynthetic rate (A) (mean ± SE) of European aspen genotypes grown under (A) control, (B) temperature, (C) UVB and (D) UVB + T.

## 4.3 Substomatal $CO_2$ (Ci)

The effect of temperature on Ci was statistically significant (Table 2). Ci was decreased by 2% under elevated temperature (Fig. 4B) and 3% under UVB + T (Fig. 4D) in comparison to the control plants (Fig. 4A). Though the main effect of UV on Ci was not statistically significant (Table 2), the interaction between UV and time was statistically significant (Table 2) and indicates the effect of UVB on Ci varied over the growing season. Moreover, there was a statistically significant difference in Ci among the genotypes (Table 2) indicating some of the genotypes (gt8, gt9, gt11 and gt1) had comparatively lower Ci than the others on an average over all the treatments (Fig. 4). The main effect of gender was not statistically significant on Ci (Table 2).

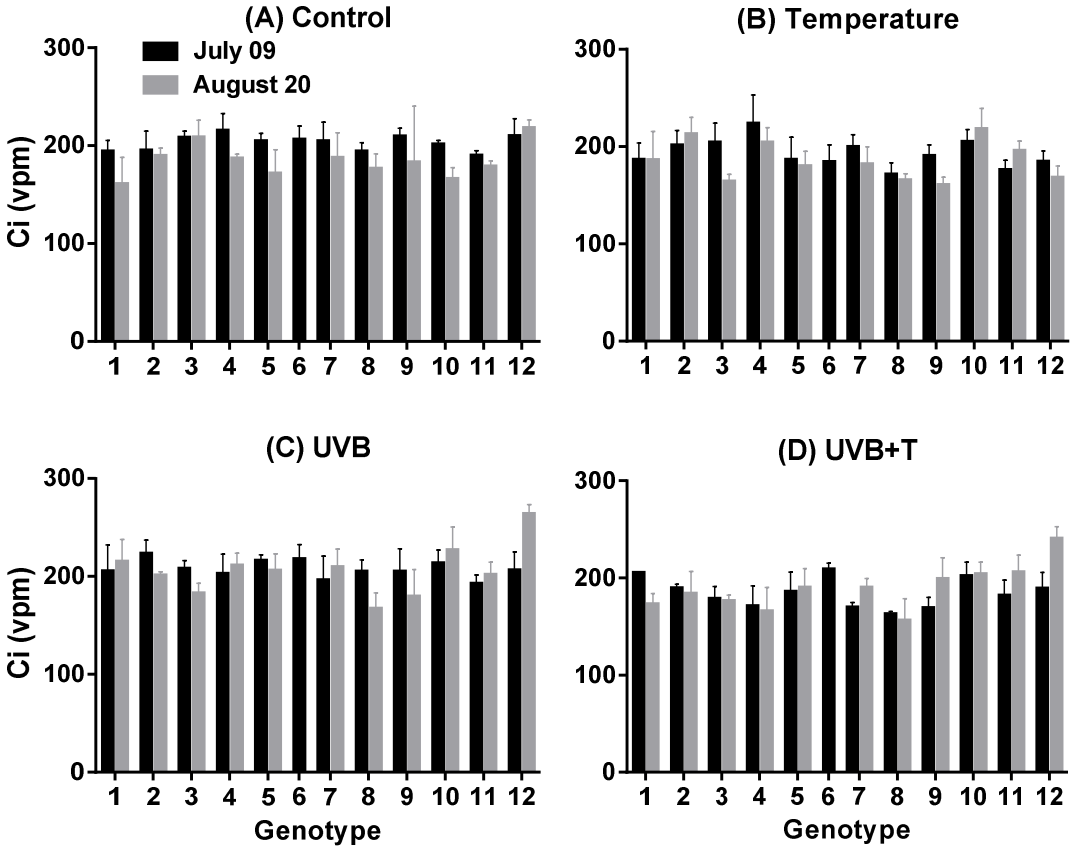


**Fig 4.** Substomatal $CO_2$ (Ci) (mean ± SE) of European aspen genotypes grown under (A) control, (B) temperature, (C) UVB and (D) UVB + T.

## 4.4 Stomatal conductance ($g_s$)

There was no statistically significant effects of temperature and UV on $g_s$ (Table 2). However, in the mid-August in contrast to early July, $g_s$ was increased by 16% under elevated temperature (Fig. 5B), and reduced by 21% under enhanced UVB (Fig. 5C) and 11% under UVB + T (Fig. 5D). Thus, the statistically significant T x Time and T x UV x Time interactions were found (Table 2). Furthermore, the effect of genotypes on $g_s$ was statistically significant (Table 2), because some of the genotypes (gt1, gt6, gt2 and gt7) had comparatively higher $g_s$ than the others when averaged over all the treatments (Fig. 5). Besides, in the mid-August as compared to early July, the variation in $g_s$ among the genotypes was increased, which results in a statistically significant Genotype x Time interaction (Table 2). Gender difference in $g_s$ was not statistically significant (Table 2).

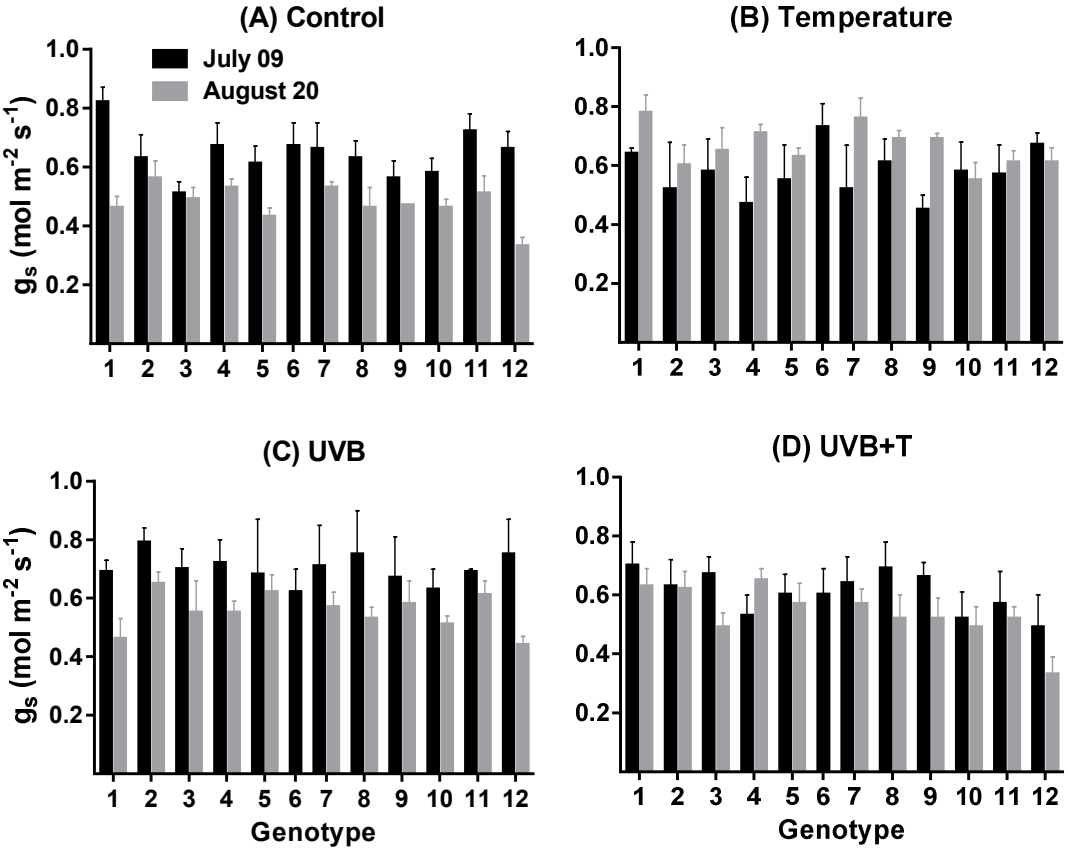


**Fig 5.** Stomatal conductance ($g_s$) (mean ± SE) of European aspen genotypes grown under (A) control, (B) temperature, (C) UVB and (D) UVB + T.

## 4.5 Transpiration rate (E)

The effects of temperature and UV on E were not statistically significant (Table 2). Even though the effect of these treatments were not significant either separately or in combination, the interaction of T x Time, UV x Time and T x UV x Time were statistically significant (Table 2). Hence, the results showed 7% enhancement of E due to elevated temperature (Fig. 6B), 9% decrease due to enhanced UVB (Fig. 6C) and 15% decrease under UVB + T (Fig. 6D) in the mid-August in comparison with early July. There were no genotype- and gender-dependent variation in E in this study (Table 2).

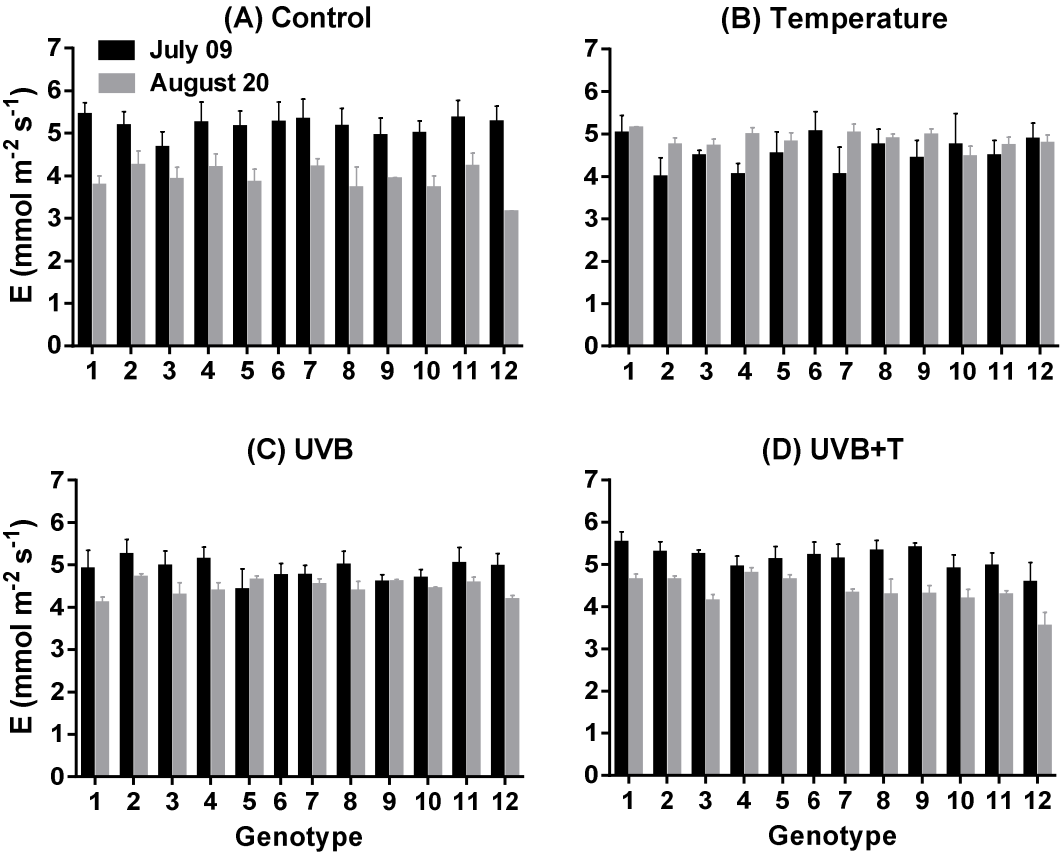


**Fig 6.** Transpiration rate (E) (mean ± SE) of European aspen genotypes grown under (A) control, (B) temperature, (C) UVB and (D) UVB + T.

## 4.6 Height growth

There was a statistically significant effect of temperature on stem height (Table 3). When compared to the control plants (Fig. 7A), stem height was 63% higher under both elevated temperature (Fig. 7B) and UVA + T (not shown in figure), and 42% higher under UVB + T (Fig. 7D). While the combined effect of UVB and temperature increased the stem height, elevated UVB negatively affected the height growth that was evidenced by a slight (4%) reduction under enhanced UVB in comparison with the control treatment. The effect of UV was not statistically significant (Table 3). The height increment was significantly higher during June-July under elevated temperature (Fig. 7B), UVA + T (not shown in figure) and UVB + T (Fig. 7D) when compared to the later part of the growing season. Thus, the interaction of temperature and time was statistically significant (Table 3). Moreover, height increment varied according to genotypes, which indicates that some of the genotypes (gt8,

gt3, gt5 and gt9) had comparatively higher stem growth rate than the others when calculated the average over all the treatments (Table 3, Fig. 7). The interaction between temperature, UV and genotypes was also statistically significant (Table 3). The variation in height growth among the genotypes was further affected by temperature alone and in combination with UVA and UVB (Fig. 7B, 7D, UVA + T is not shown in figure). Furthermore, genotypic difference in height growth was increased as the growing season advances. Thus, the interaction between genotype and time was statistically significant (Table 3). The main effect of gender was also statistically significant since male genotypes had 2% higher height growth as compared to the female genotypes (Table 3).

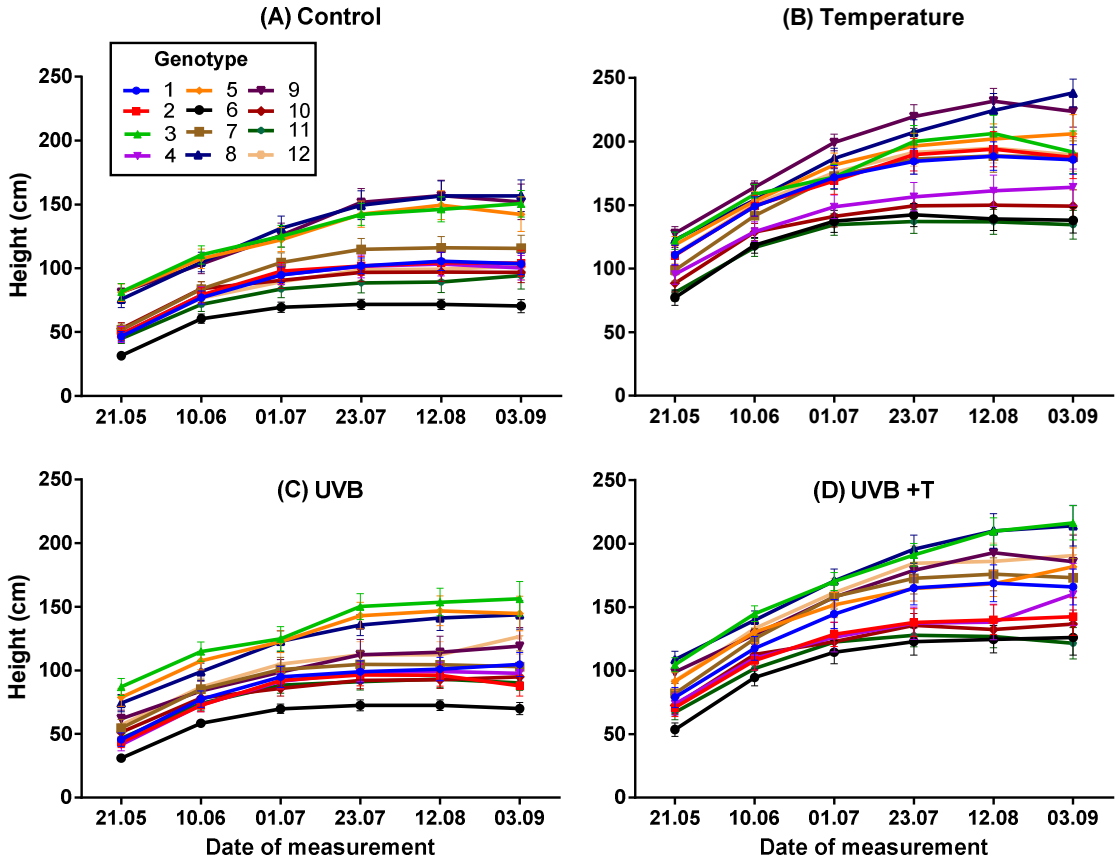


**Fig 7.** Height growth (mean ± SE) of European aspen genotypes grown under (A) control, (B) temperature, (C) UVB and (D) UVB + T.

**Table 3.** F-values obtained from the linear mixed model analysis of the effects of enhanced temperature and UV on height, basal diameter and shoot biomass of European aspen (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; MS (marginally significant), $p < 0.1$). Shoot biomass was log-transformed to meet normality assumptions.

| Effect | Height | Diameter | Shoot biomass |
|---|---|---|---|
| T | 70.751*** | 55.563*** | 96.040*** |
| UV | 1.011 | 0.956 | 2.896$^{MS}$ |
| Time | 939.395*** | 588.945*** | --- |
| T x UV | 1.210 | 0.626 | 1.037 |
| T x Time | 27.482*** | 34.672*** | --- |
| UV x Time | 0.596 | 0.504 | --- |
| T x UV x Time | 0.318 | 0.374 | --- |
| Sex | 4.703* | 0.679 | 0.503 |
| Genotype | 187.783*** | 108.687*** | 8.524*** |
| T x Genotype | 8.777*** | 11.967*** | 0.585 |
| UV x Genotype | 6.517*** | 5.793*** | 0.503 |
| Time x Genotype | 3.048*** | 0.911 | --- |
| T x UV x Genotype | 4.378*** | 2.981*** | 1.018 |
| T x Time x Genotype | 0.286 | 0.220 | --- |
| UV x Time x Genotype | 0.192 | 0.156 | --- |
| T x UV x Time x Genotype | 0.142 | 0.132 | --- |

## 4.7 Diameter growth

The effect of temperature on basal diameter growth was statistically significant (Table 3) as the basal diameter showed 44% higher increment under the elevated temperature (Fig 8B), 47% higher increment under UVA + T (not shown in Figure) and 31% higher increment under UVB + T (Fig. 8D) as compared to the control treatment (Fig 7A). In addition, temperature and time interaction effect on basal diameter was also statistically significant (Table 3). In fact, the magnitude of diameter increment was significantly pronounced during June-July under elevated temperature (Fig. 8B), UVA + T (not shown in figure) and UVB + T (Fig. 8D), and then the rate of increment was reduced by the end of the growing season. Genotypic differences also significantly influenced the basal diameter growth (Table 3). As a result, diameter growth was comparatively higher in some of the genotypes (gt9, gt3, gt5 and gt8) compared to others when averaged across the treatments (Fig. 8). Furthermore, the interaction between temperature, UV and genotype was statistically significant (Table 3). Thus, temperature alone and in combination with UVA and UVB intensified the basal diameter increment of some of the genotypes in a higher rate than the others (Fig. 8B, 8D, UVA + T is not shown in figure). There was no gender difference in diameter growth (Table 3).

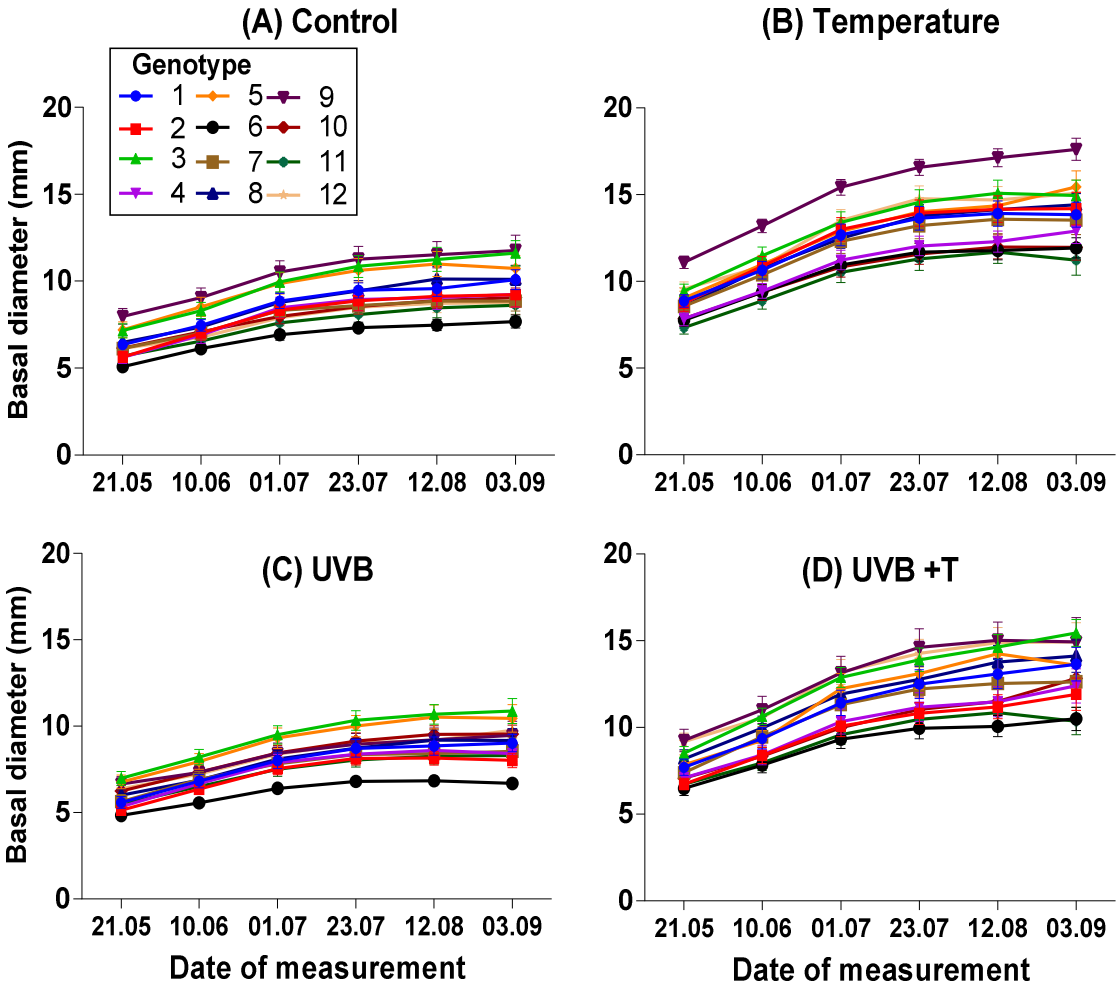


**Fig 8.** Diameter growth (mean ± SE) of European aspen genotypes grown under (A) control, (B) temperature, (C) UVB and (D) UVB + T.

## 4.8 Shoot biomass

Table 4 shows the variation in shoot biomass growth under different UV and temperature treatments. Compared to control, shoot biomass growth was 158, 242 and 109% higher under elevated temperature, UVA + T and UVB + T, respectively. As a result, the effect of temperature on the shoot biomass growth was statistically significant (Table 3). Moreover, the effect of UV on the shoot biomass growth was statistically marginally significant (Table 3). Shoot biomass was decreased by 4% under UVA, 3% under UVB, and increased by 242% under UVA+T and 109% under UVB+T when compared to the reference plants (Table 4). There was a statistically significant variation in shoot biomass growth among the genotypes

(Table 3). Therefore, gt3, gt9, gt5 and gt8 showed the comparatively higher biomass growth than the others when averaged over all the treatments (Table 4). The main effect of gender was not statistically significant on shoot biomass growth (Table 3).

**Table 4.** Total shoot biomass (g) (n = 6± SE) of European aspen genotypes grown under enhanced UV and temperature.

| Genotype | Control | T | UVA | UVA+T | UVB | UVB+T |
|---|---|---|---|---|---|---|
| gt1 | 26.87 ± 6.70 | 89.30 ± 25.37 | 22.79 ± 5.46 | 119.93 ± 39.64 | 26.02 ± 12.18 | 50.60 ± 17.82 |
| gt2 | 19.20 ± 5.33 | 80.58 ± 19.23 | 23.67 ± 6.20 | 76.32 ± 13.85 | 28.32 ± 8.63 | 35.90 ± 8.96 |
| gt3 | 35.45 ± 8.19 | 128.20 ± 30.77 | 54.00 ± 23.72 | 137.15 ± 37.31 | 42.39 ± 7.98 | 84.74 ± 22.67 |
| gt4 | 28.44 ± 11.95 | 42.07 ± 9.68 | 16.22 ± 2.54 | 105.74 ± 23.17 | 29.51 ± 14.26 | 33.83 ± 15.01 |
| gt5 | 68.02 ± 21.97 | 81.43 ± 13.89 | 36.46 ± 12.24 | 122.92 ± 27.15 | 52.59 ± 17.64 | 78.57 ± 20.10 |
| gt6 | 10.83 ± 2.24 | 37.39 ± 8.34 | 14.03 ± 5.20 | 66.54 ± 22.76 | 11.74 ± 2.12 | 20.61 ± 5.72 |
| gt7 | 21.93 ± 7.11 | 73.81 ± 25.86 | 15.45 ± 2.24 | 62.28 ± 14.94 | 20.84 ± 5.58 | 66.85 ± 18.08 |
| gt8 | 35.08 ± 15.43 | 91.73 ± 25.54 | 38.11 ± 11.57 | 103.23 ± 35.76 | 37.81 ± 22.41 | 69.34 ± 17.37 |
| gt9 | 52.35 ± 23.29 | 109.55 ± 18.55 | 38.15 ± 8.90 | 116.54 ± 16.82 | 21.57 ± 8.93 | 111.07 ± 16.34 |
| gt10 | 19.40 ± 4.81 | 47.91 ± 13.60 | 15.58 ± 2.19 | 71.36 ± 13.99 | 16.70 ± 2.65 | 26.61 ± 10.50 |
| gt11 | 17.00 ± 5.98 | 46.71 ± 9.38 | 19.23 ± 8.41 | 64.75 ± 18.93 | 23.69 ± 9.07 | 51.19 ± 9.08 |
| gt12 | 21.07 ± 5.41 | 88.44 ± 23.47 | 28.30 ± 5.83 | 87.05 ± 30.99 | 13.90 ± 5.01 | 79.06 ± 20.16 |
| Average | 29.64 | 76.43 | 26.83 | 94.48 | 27.09 | 59.03 |
| (%) changes compared to the control | --- | 157.88 | -3.67 | 241.68 | -2.69 | 108.50 |

# 5 DISCUSSION

## 5.1 Effects of elevated temperature

In the present study, chlorophyll content and gas exchange parameters were measured in early July and mid-August. Elevated temperature increased the chlorophyll content and photosynthesis, but reduced substomatal $CO_2$. Even though the increment of stomatal conductance and transpiration were not statistically significant for the whole season as a result of temperature effects, these two gas exchange parameters were however, increased significantly in mid-August under elevated temperature. Many previous studies also found the increase in chlorophyll content (Wang et al. 2003, Li et al. 2011), photosynthesis (Zhao & Liu 2009, Mäenpää et al. 2011, Hartikainen et al. 2012), stomatal conductance (Wilson & Bunce 1997, Zhao & Liu 2009, Hu et al 2014), transpiration (Wall et al. 2011, Hu et al. 2014) and decrease of substomatal $CO_2$ (Yamori et al. 2006, Zhao & Liu 2009) as a result of elevated temperature. However, several other studies reported the effects of elevated temperature on photosynthesis that are contradictory to the present findings. For example, red oak *(Quercus rubra)* seedlings were exposed to three different temperatures (ambient temperature, ambient +3°C and ambient +6°C) in the half-cylinder domed treatment chambers and revealed that both the elevated temperatures decreased net photosynthesis compared to the ambient temperature (Wertin et al. 2011). Another experiment in open-top chambers also found the decrease of net photosynthesis in scots pine (*Pinus sylvestris*) trees where the temperature was increased 2°C above the ambient level (Wang et al. 1995). The experimental methods and plant materials used may be the reasons behind the contrasting results in these reference studies. Furthermore, the optimum temperature for photosynthesis varies considerably according to species and growth conditions (Kirschbaum 2004).

Different physiological parameters may explain the temperature-induced increase in photosynthetic rate that I demonstrated in the present study. Elevated temperature increases the maximum rate of carboxylation of Rubisco (*V*cmax) and the maximum rate of electron transport (*J*max) which results in the increase of carbon assimilation rates (Way & Oren 2010). Increase in photosynthesis can also be the result of the indirect effects of elevated temperature on chlorophyll content and substomatal $CO_2$, which were significantly affected in our two-year-old European aspen plants. More favorable temperatures might ensure supply of more cytokinin from roots to leaves which in turn increases the synthesis of chlorophylls (Aiken & Smucker 1996, Zhao & Liu 2009), and increasing chlorophyll content increases

photosynthesis (Gratani & Ghia 2002, Matsumoto et al. 2005). On the other hand, temperature depended photosynthesis is influenced by the temperature dependence of substomatal $CO_2$ (Hikosaka et al. 2006). Therefore, in the present study, the reduction of substomatal $CO_2$ as a result of elevated temperature might partly explain the increase of photosynthesis under elevated temperature. Moreover, elevated temperature increased the stomatal conductance and transpiration in the mid-August in this experiment, which might have influenced the photosynthetic rate during that time as an indirect effect of elevated temperature. Elevated temperature may also increase vapor pressure deficit (VPD) (Way & Oren 2010), which might have contributed to the increase of transpiration. Higher rate of transpiration leads to the increase of photosynthetic rate as a result of $CO_2$ entering and water vapor releasing through the stomatal pores of leaves (Brodribb & Jordan 2011).

The temperature-induced increase in net photosynthesis resulted in increased height, basal diameter and shoot biomass of European aspen along with the increase of chlorophyll content and gas exchange parameters (substomatal $CO_2$ was decreased). In fact, elevated temperature affects the plant physiological and biochemical processes which facilitates carbon allocation to internal growth processes (Saxe et al. 2001, Zhao & Liu 2009, Arend et al. 2011, Hu et al. 2014). Though photosynthesis, height and basal diameter were higher under elevated temperature throughout the growth period, the magnitude of height and basal diameter increment was, however, lower at the end of summer, while the magnitude of photosynthesis was higher during the end of summer, and vice-versa. In the late summer, the decrease in the magnitude of basal diameter increment may be due to the formation of the thick walls of the late summer wood cells where a higher amount of carbon-based cellulose is needed (Tegelberg et al. 2001). The reduction in the magnitude of height and basal diameter increment in the late summer can be also from the storage impact since plants usually started reserving the photosynthates at the end of growth season to use in the winter time for respiration and early bud break in the spring (Loescher et al. 1990).

## 5.2 Effects of elevated UV radiation

Experimental studies have shown variation in plant physiological and growth performances as responses to UVB in the field conditions (Keiller & Holmes 2001, Kostina et al. 2001, Tegelberg et al. 2001, Bassman et al. 2002, Bassman & Robberecht 2006, Sedej & Gaberscik 2008, Newsham & Robinson 2009). In the present study, the main effects of elevated UVB radiation on chlorophyll content, gas exchange and growth parameters were not statistically

significant except the marginally significant effects of elevated UV radiation on shoot biomass. As this experiment was carried out in an open field with the modulated UVB radiation which might explain the negligible effects of elevated UVB radiation. Actually, it was told in earlier studies that UVB effects in a control environment is overestimated. This exaggeration effects occur in the field experiments also. Allen et al. (1999) pointed out that majority of field experiments where UVB supplementation through a 'square-wave' irradiation system is used overestimating the UVB effects. It happens because of the emission of a constant level of UVB radiation through this system, irrespective of the natural variation in solar spectrum. As a result, during cloudy days, the ratio of UVB to PPFD (photosynthetically active photon flux density) and UVA will be greater than the natural environment. On the other hand, in a modulated experiment like the one that was used in this study, supplemental UVB radiation varies according to the weather conditions which confirm the realistic ratios of UVB to PPFD and UVA. Therefore, modulated UVB radiation may be the reason of negligible effects of elevated UVB radiation in European aspen in the present study. Nybakken et al. (2012) also found the small effects of modulated UVB radiation on growth parameters of *S. myrsinifolia* in the same experimental field where the present study was carried out. They did not find the effects on height and basal diameter during the two years of experiment (2009 & 2010) while there was an additive effect of elevated UVB radiation on total biomass in the second year. In this study, statistical analysis showed the marginally significant effects of elevated UV radiation on the shoot biomass since shoot biomass was either decreased or increased under different UV treatments and under the combined treatments of UV and temperature.

## 5.3 Interactive effects of elevated temperature and UV radiation

There are very few studies available on the combined treatment of elevated temperature and UV radiation in a field situation (e.g. Day et al. 1999, Nybakken et al. 2012, Randriamanana et al. unpublished data). Day et al. (1999) and Nybakken et al. (2012) did not find any significant combined effects on the growth, chlorophyll content and reproduction . However, Randriamanana et al. (unpublished data) found the significant interactive effects on the height, basal diameter and shoot biomass of European aspen during the first year of experiment where supplemental UVB radiation acted negatively against the positive warming effects. In this study, statistical analysis showed no significant effects of the combined treatment of elevated UV and temperature on any of the studied parameters. However,

elevated UVB radiation partially counteracted the stimulation effects of temperature in almost every parameter studied. For instance, CCI was 28% higher under elevated temperature and 22% higher when combined with elevated UVB radiation in comparison with control. Stem height was increased by 63% under elevated temperature whereas 42% under UVB + T as compared to the control. Moreover, these two climatic factors produced significant results combined with the time since the interactive effects of T x UVB x Time were statistically significant on photosynthesis, stomatal conductance and transpiration rate. Actually, in the mid-August, these three gas exchange parameters were further increased under elevated temperature as compared to early July. Under elevated UVB radiation, these parameters were decreased in the mid-August though the main effects of elevated UVB radiation on these parameters were not statistically significant. In a field condition, plants experience unpredictable variations in light, temperature, humidity, soil moisture and wind (Jänkänpää et al. 2012), and these factors varies as a function of time. Therefore, apart from the experimental warming and UVB radiation, other natural environmental factors might influence the photosynthesis, stomatal conductance and transpiration, and which might produce the fluctuation of results from the early July.

## 5.4 Genotype effects

In this study, it is evident from the graphs that there is a significant genotypic variation in chlorophyll content, gas exchange and growth parameters under every treatments. Statistical analysis also revealed the significant variation among the genotypes in all the studied parameters except the transpiration rate irrespective of treatments. Among the twelve genotypes used in the present study, seven (gt1, gt5, gt6, gt7, gt10, gt11 and gt12) were collected from Eastern Finland and the others (gt2, gt3, gt4, gt8 and gt9) were collected from Southern Finland. Hence, there was an obvious climatic differences including solar irradiation, humidity, temperature, wind etc. among the origins of these genotypes. Therefore, differences in the origins of genotypes may account for this genotypic variation. These results are accordance with the findings of previous studies where they have recorded the variation in chlorophyll content (Rowland 2001), gas exchange (Rowland 2001, Hartikainen et al. 2012, Possen et al. 2014) and growth parameters (Turtola et al. 2006, Possen et al. 2011) among the genotypes of broad leaf tree species in common garden experiments.

Apart from the genotypic variation, dioecious species shows the gender differences in physiology, morphology and growth (Gehring & Monson 1994, Liebig et al. 2001,

Randriamanana et al. 2014b). In the present study, half of the genotypes (gt1-6) were females and half of them (gt7-12) were males. Among the studied parameters, statistically significant gender effects were found only in cholorophyll content and height growth. Female genotypes had higher chlorophyll content while male genotypes had higher height growth. In case of dioecious species, the behavior of resource use is different in males and females (Nybakken et al. 2012). Female individuals invest greater amount of resources for the reproduction whereas their male counterparts allocate greater amount for the growth increment (Montesinos et al. 2006, Cepeda-Cornejo & Dirzo 2010). In this study, inherent greater growth tendency may be the reason of comparatively higher height growth of male genotypes (Randriamanana et al. 2014b).

In the present study, one of the notable features is that some of the genotypes (gt3, gt5, gt8 and gt9) showed comparatively higher performance in height, basal diameter and shoot biomass growth than the others when averaged over all the treatments. Parent materials of gt3, gt8 and gt9 were collected from Southern Finland. In a particular season, the amount of growth depends on how many days growth continues and the mean daily growth rate of that growth period, and the duration of growth period depends on the experimental location and origin of the parent material (Emhart et al. 2006). Lower-latitude genotypes are used to grow for a longer growth period than the higher-latitude ones. Therefore, when grown at higher latitudes, genotypes from the Southern Finland (60º N) might have higher inherent growth and grew for a longer period as compared to the genotypes of Eastern Finland (62º N) which results in a higher height, diameter and aboveground biomass growth.

It is mentioned above that the combined elevated UV and temperature had no effects on any of the studied parameters. However, these two climatic factors combined with genotype showed the significant impact on height and basal diameter growth. Thus, some of the genotypes were further affected by elevated temperature and UV radiation in height and basal diameter growth. For example, when calculated the average across the treatments, height and basal diameter of gt9 were 141.46 cm and 11.69 mm, respectively. However, its height was 194.31 cm under elevated temperature, 95.86 cm under elevated UVB radiation, and 157.14 cm under UVB + T. Similarly, its basal diameter was 15.17 mm, 8.19 mm and 12.99 mm under elevated temperature, elevated UVB radiation and UVB + T, respectively. Therefore, the results are accordance with my initial hypothesis that to some extent, genotypic responses of some parameters were more pronounced under elevated UVB and temperature. Earlier studies also found genotypic variation in height and basal diameter

growth under elevated temperature and UV radiation in broad leaf tree species (Tegelberg et al. 2003, Pulkkinen et al. 2013).

# 6 CONCLUSIONS

In this study, single elevated temperature effects were positive for the physiological and growth performances of European aspen. Although the main effects of elevated UV radiation were not significant in most of the studied parameters, but the effects were detrimental to some extent. Thus, in a combined treatment of elevated temperature and UV radiation, the parameter values were comparatively smaller than the single temperature effects. However, these values are still far higher as compared to the control plants. Therefore, it can be said from the view point of this study that broad leaf tree species in the boreal region will gain a substantial benefit in the future climate scenario since the elevated $CO_2$ effects are also additive for tree growth in this region.

This study also revealed the significant genotypic variation in almost all the parameters studied. Exploring genotypic variation helps to select the best genotype for the future. Genotypes from Southern Finland showed higher performance in growth as compared to the Eastern ones. Superiority in physiology and growth of some of the genotypes were again provoked by the elevated temperature and UVB radiation. In future, experiments with more European aspen genotypes may produce more accurate information about the genotypic variation and also the variation related to regions.

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